



Autopsy-based morphometric study of coronary atherosclerosis in young adults

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Background & objectives: The burden of cardiovascular diseases is high in Kerala, India, and a considerable proportion of these occur in young people. The objective of this study was to estimate the severity of atherosclerosis in autopsies done for accidental and suicidal deaths in victims below 40 yr of age.

Methods: Coronary arteries from 77 autopsies done for unnatural deaths in a population below 40 yr were graded, and the degree of stenosis, intimal thickness index (ITI) and the intima-media ratio (IMR) were measured.

Results: There were 65 males and 12 females in the sample. The American Heart Association (AHA) type 3-6 (pathological intimal thickening) was seen in 55.4 per cent [95% confidence interval (CI): 42.5-67.7%] of males and 25 per cent (95% CI: 5.5-57.2%) of females and advanced lesions (type 4-6) in 44.6 per cent (95% CI: 32.3-57.5%) of males and 8.3 per cent (95% CI: 0.2-38.5%) of females. Types 5 or 6 lesions were seen in 32.2 per cent (95% CI: 21.2-45.1%) of males. The mean stenosis was 57.3 per cent in males and 40.6 per cent in females. More than 40 per cent stenosis was seen in 76.6 per cent cases, more than 50 per cent in 54.5 per cent cases and more than 75 per cent stenosis in 14.3 per of the sample. The mean ITI (MIT) was 1.85 and the mean IMR was 4.11. The degree of stenosis, MIT and IMR were significantly associated with male sex, overweight and smoking.

Interpretation & conclusions: Morphometric data showed that the degree of atherosclerotic narrowing of coronary arteries in young non-diseased population was high. It portends a danger to the community unless preventive measures are taken up.

Key words Atherosclerosis - autopsy - coronary artery disease - IMR - ITI - morphometry - young adults

The overall age-adjusted prevalence of definite coronary artery disease (CAD) is 4.8 per cent in men and 2.6 per cent in women in Kerala, India. There was almost a three-fold increase since 1993¹. In the PROLIFE (Population Registry of Lifestyle Diseases)

study conducted in Kerala, the standardized death rates for cardiovascular diseases (CVDs) in the one revenue block were 490 for men and 231 for women per 100,000 person-years². An earlier hospital based study showed a good proportion of heart attacks in

young individuals below 40 yr of age in Kerala³. We undertook this study to record the actual morphometric measurements of the artery in autopsy material of unnatural deaths occurred in a hospital in Kerala during a year.

Material & Methods

The study was conducted in the departments of Pathology and Forensic Medicine, Government TD Medical College, Alappuzha, Kerala, during July 2012 to June 2013. All cases autopsied for deaths due to accidents or suicides in those below 40 yr during the study period were included. Those having previously diagnosed ischemic heart diseases, were excluded. The study protocol was approved by the institutional ethics committee.

Relevant clinical data were obtained from the nearest relative of the deceased or the persons accompanying the body, using an open-ended questionnaire which included history regarding any previous illness in the deceased with special reference to heart disease, cerebrovascular disease, diabetes, hypertension and peripheral vascular disease. The personal habits of the deceased, especially related to smoking and alcohol consumption, were also recorded. General examination during autopsy including height and weight were recorded. Aorta was opened longitudinally, and gross examination of the ascending aorta, arch of the aorta and descending aorta was done. All the three coronaries were examined at 5 mm intervals and sections for histopathological examination were taken from area where there was greatest narrowing. The thickness of the left and right ventricles was measured.

Histology: Sections were stained by haematoxylin and eosin and Verhoeff-Van Gieson (VVG). The atheromatous lesions were graded on a six-point ordinal scale as per the American Heart Association (AHA) guidelines⁴. Medial changes such as thinning of media, calcification and inflammatory cell infiltration were recorded. The type and amount of inflammatory cells in the adventitia and periadventitial fibrosis were also noted.

Morphometric measurement: To take the morphometric measurements, microscopic images of the VVG-stained slides were captured on an Olympus BX43 microscope (Olympus Corporation, Tokyo, Japan) with camera and morphometric measurements made with cellSens imaging software (Olympus Corporation,

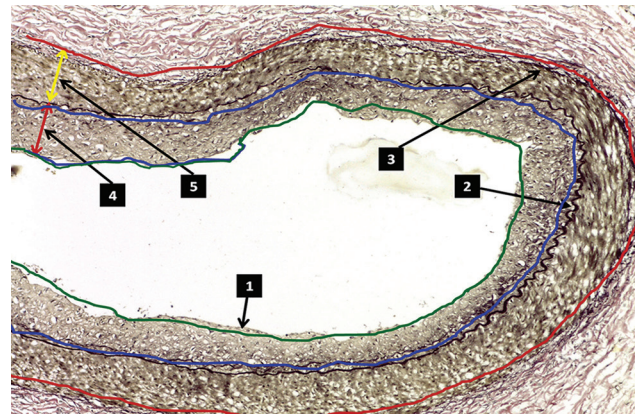


Fig. 1. Images from Verhoeff-Van Gieson-stained sections ($\times 40$) of coronary were used for morphometry using the cellSens software (Olympus Corporation, Tokyo, Japan). (1) Luminal border, (2) internal elastic lamina, (3) external elastic lamina, (4) intimal thickness, (5) medial thickness. The inner green line marks the luminal outline, the middle blue marks the internal elastic lamina and the outer red marks the external elastic lamina for measurement of areas.

Tokyo, Japan). In each section, the following variables were measured (Fig. 1), as detailed in Ruengsakulrach *et al*⁵. (i) luminal area (LA); (ii) internal elastic lamina area (IELA) which is the area encompassed by the internal elastic lamina; (iii) external elastic lamina area (EELA) which is the area encompassed by the external elastic lamina; (iv) width of the intima at maximal intimal thickness; and (v) width of the media at maximal intimal thickness. The intimal area was calculated by subtracting the LA from the IELA and medial area by subtracting the IELA from the EELA area.

Measures for assessing severity of atherosclerosis in the coronaries included (i) percentage of luminal narrowing = $(\text{intimal area} / \text{IEL area}) \times 100$, (ii) intimal thickness index (ITI) = $\text{intimal area} / \text{medial area}$, and (iii) intima-to-media ratio (IMR) = $\text{width of intima at maximal intimal thickness} / \text{width of media at maximal intima thickness}$.

Statistical analysis: Data were entered in Open Office spreadsheet and analyzed using Epi Info statistical software (Centers for Disease Control and Prevention, USA). Statistical tests employed were Chi-square test for proportions, *t* test for means of two independent variables and ANOVA for means of more than two variables. In case of *t* test and ANOVA, equality of variance assumption was tested by the Bartlett's test and Kruskal-Wallis test applied when the variances were not homogenous.

Results

A total of 77 autopsies were done (65 males, 12 females). The mean age of the sample was 30.3 [95% confidence interval (CI): 28.6-32.0] yr and the median 32 yr. The average body mass index (BMI) was 22.6 kg/m² (95% CI: 21.8-23.4), with 27.7 per cent of males and 16.7 per cent of females being overweight (BMI more than 25 kg/m²). About 53.8 per cent of males were smokers. Traffic accidents (n=30, 39%), hanging (n=25, 32.5%), poisoning (n=9, 11.7%), drowning (n=6, 7.8%), homicide (n=4, 5.2%), electrocution (n=2, 2.6%) and burns (n=1) were the causes of death in the 77 cases studied.

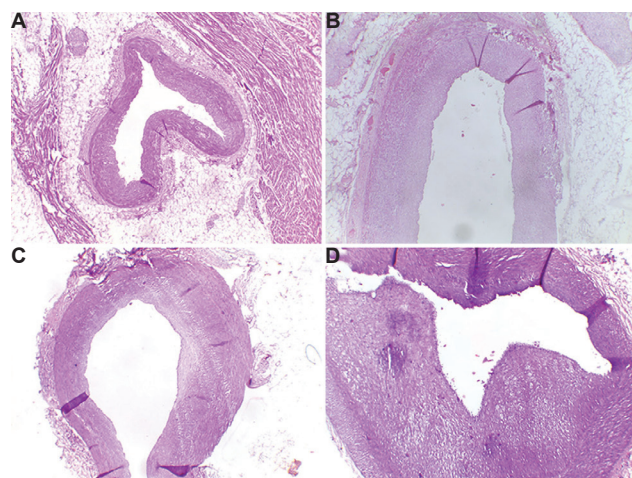


Fig. 2. Representative sections of coronary arteries: Normal and American Heart Association (AHA) type I-III. (A) Normal coronary artery, (B) AHA type I lesion, (C) AHA type II lesion, (D) AHA type III lesion (H and E, $\times 40$).

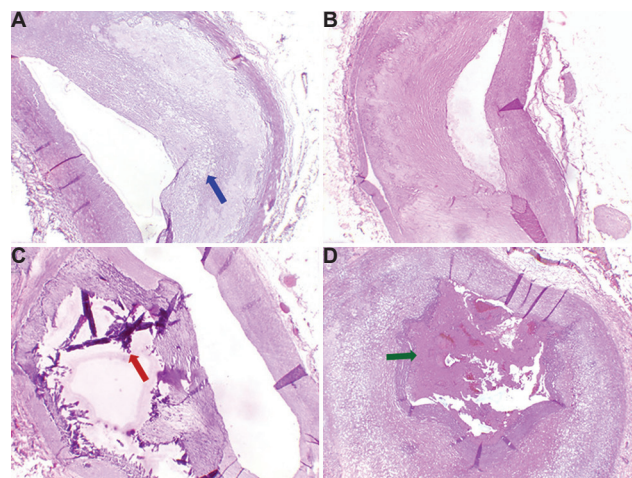


Fig. 3. Sections of coronary arteries: American Heart Association (AHA) type IV-VI. (A) AHA type IV lesion, (B) AHA type Va lesion with lipid core (Blue arrow), (C) AHA type Vb lesion with calcification (red arrow), (D) AHA type VI lesion with thrombus (green arrow) (H and E, $\times 40$).

The arteries with the maximum narrowing sampled were left anterior descending in 49.4 per cent (n=38), right coronary in 28.6 per cent (n=22) and circumflex in 22.1 per cent (n=17) of the cases. The grading of the coronary arteries according to the AHA classification is shown in Table I, and the representative sections of some of the AHA types are shown in Figures 2 and 3. Among the 21 cases of type V lesions, nine were type Va and 12 type Vb. The sole type VI case showed a thrombus (type VIc) (Fig. 3).

The presence of AHA type III and upward [corresponding to pathological intimal thickening (PIT)] and its relation to different variables is shown in Table II.

Table I. Grading of coronaries in the sample according to American Heart Association classification⁴ and sex

Type	Male, n (%)	Female, n (%)	Total, n (%)
Normal	5 (7.7)	5 (41.7)	10 (13.0)
I	18 (27.7)	3 (25.0)	21 (27.3)
II	6 (9.2)	1 (8.3)	7 (9.1)
III	7 (10.8)	2 (16.7)	9 (11.7)
IV	8 (12.3)	0 (0.0)	8 (10.4)
V	20 (30.8)	1 (8.3)	21 (27.3)
VI	1 (1.5)	0 (0.0)	1 (1.3)
Total	65 (100.0)	12 (100.0)	77 (100.0)

Table II. Frequency of American Heart Association types III, IV, V, VI (corresponding to pathological intimal thickening) according to different variables

Variable	n	Per cent of cases (95% CI)	<i>P</i>
Sex			
Female	3	25.0 (5.5-57.2)	0.0530
Male	36	55.4 (42.5-67.7)	
Age group (yr)			
11-20	2	25.0 (3.2-65.1)	0.2320
21-30	14	48.3 (29.5-67.5)	
31-40	23	57.5 (40.9-73.0)	
Smoking			
No	18	42.9 (27.7-59.0)	0.1340
Yes	21	60.0 (42.1-76.1)	
Overweight			
No	23	40.4 (27.6-54.2)	0.0022
Yes	16	80.0 (56.3-94.3)	
Whole sample	39	50.7 (39.0-62.2)	-

CI, confidence interval

Table III. Mean coronary stenosis, intimal thickness index (ITI) and intima-media ratio (IMR) according to different variables

Variable	Stenosis Mean (95% CI)	<i>P</i>	ITI Mean (95% CI)	<i>P</i>	IMR Mean (95% CI)	<i>P</i>
Sex						
Female	40.6 (36.2-45.0)	0.001	1.07 (0.75-1.39)	0.027	2.05 (1.12-2.99)	0.006
Male	57.3 (52.9-61.7)		1.95 (1.60-2.31)		4.48 (3.64-5.33)	
Age group (yr)						
11-20	41.3 (31.2-51.2)	0.085	0.95 (0.62-1.28)	0.071	1.40 (0.89-1.92)	0.004
21-30	56.1 (49.4-62.8)		1.86 (1.36-2.36)		4.0 (3.06-4.95)	
31-40	56.4 (50.9-61.9)		1.96 (1.50-2.41)		4.72 (3.52-5.92)	
Smoking						
No	48.7 (43.3-54.1)	0.001	1.46 (1.09-1.84)	0.001	2.99 (2.23-3.74)	0.001
Yes	61.9 (56.8-67.0)		2.24 (1.75-2.41)		5.45 (4.21-6.69)	
Overweight						
No	51.4 (46.8-56.2)	0.005	1.65 (1.28-2.02)	0.0020	3.57 (2.75-4.38)	0.0051
Yes	63.9 (57.4-70.4)		2.28 (1.77-2.79)		5.64 (4.11-5.92)	
Whole sample	54.7 (50.7-58.7)	-	1.85 (1.61-2.09)	-	4.11 (3.36-4.85)	-

Table IV. Presence of coronary artery stenosis $\geq 40\%$ according to different variables

Variable	Proportion of those with stenosis $\geq 40\%$ with (95% CI)	<i>P</i>
Sex		
Female	50.0 (21.1-78.9)	0.027
Male	81.5 (70.0-90.1)	
Age group (yr)		
11-20	50.0 (15.7-84.3)	0.139
21-30	75.9 (56.5-89.7)	
31-40	82.5 (67.2-92.7)	
Smoking		
No	64.3 (48.0-78.5)	0.005
Yes	91.4 (76.9-98.2)	
Overweight		
No	70.2 (56.6-81.6)	0.024
Yes	95.0 (75.1-99.9)	
Whole sample	76.6 (65.6-85.5)	-
CI, confidence interval		

Almost half ($n=39$, 50.6%) of the samples had PIT. It was significantly high in those who were overweight. The proportion of those with advanced lesions (AHA 4, 5 and 6) was 39 per cent overall and 44.6 per cent in males. Table III shows mean coronary stenosis, ITI and IMR according to different variables. Table IV shows presence of more than 40 per cent stenosis according to

different variables; 76.6 per cent sample showed more than 40 per cent stenosis. It was significantly higher in male sex, smokers, and overweight patients ($P<0.05$). Age groups showed no relation to PIT (AHA 3-6).

Discussion

More than 40 per cent stenosis was observed in about two third of sample. Studies done in the middle of the previous century showed that the coronary lesions were fewer and milder among Indians when compared to the developed countries⁶⁻⁸. In our sample, significant PIT and type 5+6 lesions were seen in 50.6 and 28.6 per cent samples, respectively. These were relatively high figures for a young population of India. Other autopsy studies from India showed a similar pattern, though the methodologies followed were not well defined, and in the absence of morphometric measurements, these were not helpful for comparisons⁹⁻¹¹.

Coronary arteries have been studied in American combat casualties during the Korean and Vietnam wars in the 1950s and 1970s, respectively^{12,13}. Other autopsy studies in young populations from the United States also reported the degree of stenosis^{14,15}. A comparison of coronary stenosis from these studies with the current study is given in Table V.

A study from Spain in 2003¹⁷ in the 12-35 yr age group dying of external causes found type IV lesions

Table V. Proportion of cases having varying degrees of coronary artery stenosis in various studies (%)

Study	Population	>40% stenosis (%)	>50% stenosis (%)	>75% stenosis (%)
Virmani <i>et al</i> ¹⁶	Korean war (US casualties) Mean age 20.5 yr	-	19	6.4
MacNamara <i>et al</i> ¹³	Vietnam war (US casualties) Mean age 22.1 yr	-	-	5
Joseph <i>et al</i> ¹⁴	Young trauma victims (US) <35 yr	-	20.7	9
McGill <i>et al</i> ¹⁵	Young trauma victims (US) 30-34 yr	19 (men) 8 (women)		
Current study	Accidents, suicide <40 yr	76.6	54.5	14.3

in 34 per cent of men and 0 per cent of women. The prevalence increased with age and was nearly 60 per cent in the 30-35 age group. However, there were no type V or VI lesions in their sample. In our study, 30.8 per cent of men and 8.3 per cent of women had AHA type V lesions. Severity of lesions was found to be related to BMI, male sex and smoking habits in our study. Lipid profiles could not be estimated because of non-availability of samples.

To conclude our study showed AHA types 3-6 PIT in about half of the autopsy samples of young individuals studied. More than 40 per cent stenosis was seen in >75 per cent cases. Urgent preventive measures need to be taken to stop these atherosclerotic changes in the coronaries of young adults.

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Conflicts of Interest: None.

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